Identification of Quantitative Trait Loci Associated with Fructose, Glucose, and Sucrose Concentration in Snap Bean Pods

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ABSTRACT

Sugars, including fructose, glucose, and sucrose, contribute significantly to the flavor and consumer acceptance of snap beans (*Phaseolus vulgaris* L.). Differences between dry and snap bean cultivars and among snap bean cultivars in the patterns of accumulation of sugars have been observed. In 'Eagle', a white-seeded, large-sieve snap bean cultivar, fructose and glucose concentrations in developing pods decreased while sucrose concentration increased with increasing pod size. In contrast, fructose and glucose concentrations increased while sucrose remained unchanged with increasing pod size in Puebla 152, a black-seeded dry bean landrace from Mexico. A population derived from the cross Eagle × Puebla 152 consisting of 75 $F_{9:10}$ recombinant inbred lines (RILs) was developed by single seed descent. Significant differences in fructose, glucose, and sucrose concentrations of sieve size 4 (8.33–9.52 mm) pods were observed among RILs. No significant genotype × year interactions were observed. Heritability estimates for fructose, glucose, and sucrose were 0.85 ± 0.16, 0.81 ± 0.16, and 0.85 ± 0.16, respectively. A single quantitative trait locus (QTL) on linkage group B1 was identified that is closely linked to random amplified polymorphic DNA (RAPD) marker W9.1050 and explains 28.8 and 26.6% of the variation in pod fructose and sucrose concentration, respectively. A two-QTL model, including W9.1050 and RAPD marker F8.500 on linkage group B6, explained 36.4% of the variation in glucose concentration in pods.

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Abbreviations: EP-RIL, Eagle × Puebla 152 recombinant inbred line; HPLC, high-performance liquid chromatography; LOD, logarithm to the base 10 of the likelihood odds ratio; LSMEAN, least square mean; pLOD, penalized logarithm to the base 10 of the likelihood odds ratio; QTL, quantitative trait loci/locus; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred line.

Fructose, glucose, and sucrose in many fruits and vegetables are important components of flavor, with sweeter varieties having increased consumer preference (Auerswal et al., 1999; Malundo et al., 1995; Merrow and Hopp, 1961). Sugars can also mask undesirable flavors associated with S-containing compounds, such as glucosinolates, and promote consumption (Schonhof et al., 2004). In legume crops, including common bean (*Phaseolus vulgaris* L.), soybean [*Glycine max* (L.) Merr.], and pea (*Pisum sativum* L.), both quality and preference of pods and seeds are positively correlated with increased sugar concentration (Martens, 1986; Mkanda et al., 2007; Wszelaki et al., 2005).

In snap beans, the primary source of C is sucrose transported into the pod through the phloem where it is cleaved into the monosaccharides glucose and fructose to be used for cell growth, respiration, and storage molecule formation (Weber et al., 1997, 2005). The glucose and fructose used in pod growth are the primary products of acid invertase activity. In large sieve snap bean varieties, the activity of acid invertase peaks near the intermediate stage of pod development

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(Sung et al., 1994). As pod growth continues, acid invertase activity declines (Sung et al., 1994), fructose and glucose concentrations decrease, and sucrose concentrations increase (VandenLangenberg, 2011). In contrast to snap beans, in the dry bean Puebla 152, fructose and glucose concentrations continue to increase with increasing pod diameter and sucrose concentration remains unchanged (VandenLangenberg, 2011). In vegetable soybean seed the activity of the cell-wall bound fraction of acid invertase was shown to decline throughout development and higher levels of activity were negatively correlated with sucrose and positively correlated with fructose and glucose contents (Sitthiwong et al., 2005).

Quantitative trait loci (QTL) associated with sugar concentration and flavor-related compounds have been identified in several fruits and vegetables. In super-sweet corn (*Zea mays* L.), 11 QTL were identified explaining 63.7% of the total variance in soluble sugar concentration (Qi et al., 2009). Similarly, 17 QTL that account for 53% of the total variance in sucrose concentration were identified in soybean (Maughan et al., 2000). In tomato (*Solanum lycopersicum* L.), QTL for 15 different traits, including sugar concentration and flavor as assessed by a taste panel, were identified (Fulton et al., 2002).

In common bean, numerous QTL have been identified including those associated with disease and insect resistance, seed size and color, storage proteins, and pod color (Kelly et al., 2003). Quantitative trait loci associated with seed micronutrients, including Zn, Fe, P, and phytic acid, have also been identified (Cichy et al., 2009). Differences between dry and snap beans as well as variation among snap bean cultivars in the pattern of sugar accumulation in developing pods have been observed (VandenLangenberg, 2011). In spite of the importance of sugar concentration in flavor, the genetic mechanisms controlling sugar concentration in snap beans have not been identified.

Pods of Eagle, a large-sieve snap bean, have decreasing monomer and increasing disaccharide concentrations with increasing pod size while pods of Puebla 152, a dry bean, have a contrasting sugar accumulation profile (VandenLangenberg, 2011). The genetic basis for these differences in sugar content is poorly understood and markers that might be used to breed for more desirable sugar contents are not available. Identification of QTL associated with the concentration of the monosaccharides fructose and glucose and the disaccharide sucrose will assist the breeder in efforts to modify sugar concentration in snap bean pods. The objective of this study was to identify QTL associated with variation in fructose, glucose, and sucrose concentration in an Eagle × Puebla 152 recombinant inbred line (EP-RIL) population.

MATERIALS AND METHODS

Plant Material

A recombinant inbred line(RIL) population derived from a cross between Eagle, an Andean large sieve snap bean variety developed in 1971 by Asgrow Seed Co. (presently Monsanto Co.), and Puebla 152, a black-seeded dry bean landrace from Mexico, was used to identify QTL for pod glucose, fructose, and sucrose concentration. The EP-RIL population consisted of 75 $F_{9:10}$ lines developed by single seed descent (Skroch, 1998). A seed increase of each RIL to $F_{10:11}$ was performed in greenhouses at the University of Wisconsin ARS at West Madison, WI, between the 2009 and 2010 field trials.

Plant Culture and Sampling

In the summer of 2009 and 2010, 75 $F_{9:10}$ and $F_{10:11}$ lines of the EP-RIL, respectively, were direct seeded in 91-cm rows spaced 76 cm apart at the University of Wisconsin ARS at West Madison, WI, using a randomized complete block design with two replications. To facilitate timely harvesting of pods, replicates were planted approximately 1 wk apart each year. In 2009, complete blocks were planted on 26 June and 3 July, and in 2010, plantings of complete blocks occurred on 25 June and 3 July. Soil at the University of Wisconsin ARS is classified as a Plano Silt Loam and standard cultural practices were followed (Bussan et al., 2012).

Pods of 74 of the 75 EP-RILs were harvested corresponding to sieve size 4 (8.33–9.52 mm) (Quintana et al., 1996; Robinson et al., 1963). Sieve size was measured 90° off the suture at the center of the pod. A sample of five pods for each plot was harvested at random and bulked. All pods were harvested in the early morning and immediately placed in a cooler that contained ice. Pods remained on ice for approximately 3 hr and then were stored at −80°C and freeze dried before sugar extraction.

Alcohol-Soluble Sugar Extraction

An ethanol extraction method followed by high-performance liquid chromatography (HPLC) can be used to reliably and quickly measure soluble sugar concentration in snap bean pods (Lopez-Hernandez et al., 1994; Sánchez-Mata et al., 2002). Samples were lyophilized in a VirTis Freezemobile 25 (VirTis Co., Inc.) and ground to a fine powder in an industrial paint shaker with metal beads. Alcohol soluble solids were extracted twice for 24 h each using 5.0 mL of 80% ethanol at 60°C from 0.1 g of powdered sample. Pooled extracts were brought up to a total volume of 10 mL with additional 80% ethanol. Extract samples of 1.0 or 1.5 mL were dried under vacuum without heating and resuspended in 1 mL ultrapure water. Resuspended samples were passed through a previously washed (5 mL methanol followed by 5 mL water) Sep-Pak C18 cartridge (Waters Associated, Inc.). Samples were then filtered through a 0.2 μm Millipore membrane (Corning Inc.).

Samples (10 μl) were analyzed with a Shimadzu Prominence HPLC (Shimadzu Corp.) using a 300 by 7.8 mm Rezex ROA-organic acid column (Phenomenex, Inc.) with 0.0041% HPLC grade formic acid (pH 3.30 ± 0.02) (Sigma-Aldrich Corp.) in water as the mobile phase at a flow rate of 0.59 mL min^{-1} and a Shimadzu refractive index detector (Shimadzu Corp.). Peaks were identified and quantified based on characteristic retention times and peak area relative to authentic standards.

Statistical Analysis of Field Data

Data was analyzed using the statistical packages JMP 8.0.1 (SAS Institute, 1994), R (R Development Core Team, 2008), and R/qtl (Broman et al., 2003). Analysis of variance (ANOVA) and

QTL analyses were performed on the EP-RIL population for pod glucose, fructose, and sucrose concentration using normalized least square means (LSMEANs) obtained from the Box-Cox family of transformations (Box and Cox, 1964). Year and sieve size were treated as fixed variables. Correlations between glucose, fructose, and sucrose were estimated using a restricted maximum likelihood method.

Variance Components and Heritability Estimates

Variance components for the ANOVA were computed from the mean square errors from the ANOVA for transformed values of pod glucose, fructose, and sucrose concentration in the EP-RIL population according to the expectations for random models (Cockerham, 1963; Knapp et al., 1985). Narrow-sense heritability and standard error estimates were calculated on a progeny mean basis using Hallauer and Miranda (1988) procedures. Heritability estimates computed from inbred lines are considered narrow-sense estimates because the dominance variance and covariance of additive and dominance effects are equal to zero in the $F_{9:10}$ and $F_{10:11}$ EP-RIL populations (Cockerham, 1983). Using these assumptions, the genetic variance for the lines is equivalent to the additive variance and the additive × additive interactions. Epistatic relationships of additive effects may cause an overestimation of narrow-sense heritability (Cockerham, 1983).

Identification of QTL in the Eagle × Puebla 152 RIL Population

Genotypic data from the Ph.D. thesis of F.M. Navarro (2005) for the EP-RIL consisting of 153 random amplified polymorphic DNA (RAPD) markers was used for this study. The marker order from a previously published linkage map for the EP-RIL was used to reconstruct the linkage groups and estimates of genetic distance (Skroch, 1998). The new molecular map consists of 11 linkage groups spanning 860.9 cM with an average spacing between markers of 6.1 cM and a maximum of 30.8 cM (VandenLangenberg, 2011).

The focus of the QTL identification was to identify putative QTL associated with fructose, glucose, and sucrose pod concentrations and estimate additive effects. An exploratory approach to QTL mapping was used to select the best model explaining the observed phenotypic variance. Genome-wide thresholds to establish significant ($p < 0.05$) logarithm to the base 10 of the likelihood odds ratio (LOD)-score values were calculated using permutation tests ($n = 1000$) in the R package R/qtl (Broman et al., 2003) for single- and two-QTL models for each pod phenotype, including fructose, glucose, and sucrose concentrations. Significant LOD-score thresholds values equaled 2.86, 2.61, and 2.80 for single-QTL models for fructose, glucose, and sucrose, respectively. Standard interval mapping using Haley-Knott regression was performed for single- and two-QTL models to establish potential candidate QTL, additive QTL, and QTL × QTL interactions. Based on these results, QTL models for each phenotype were compared using penalized LOD (pLOD) scores calculated as

$$\mathrm{pLOD}\alpha(\gamma) = \mathrm{LOD}(\gamma) - \mathrm{T}\ |\gamma|,$$

in which γ denotes a model, $|\gamma|$ is the number of QTL in the model, and T is a penalty on the size of the model (Broman and Sen, 2009). Chosen models were compared to the results of a forward selection, backward elimination multiple regression approach.

RESULTS AND DISCUSSION

Sugar Concentration in Pods in the Eagle × Puebla 152 RIL Population

Significantly higher concentrations of fructose and significantly lower concentrations of sucrose were observed in pods evaluated in 2010 compared to 2009 (Table 1). No significant differences between years were observed for glucose. Significant variation for fructose, glucose, and sucrose pod concentration was observed among the EP-RILs in 2009 and 2010 for all three sugars (Table 1). Fructose, glucose, and sucrose pod concentrations ranged from 5.87 to 104.36, 2.97 to 50.69, and 1.08 to 18.61 mg g^{-1} dry weight based on raw values, respectively (data not shown). No year × genotype interactions were observed in pod fructose, glucose, and sucrose concentrations. The mean squares associated with the genetic component of variation are 10 to 70 times higher than the variances attributable to the genotype × year interaction (Table 1). High heritabilities were estimated for the transformed concentrations in all three sugars, ranging from $h^2 = 0.85$ to $h^2 = 0.81$ (Table 1). The high heritability for sugar concentration and significant correlations among sugars suggest that breeding for increased concentration of specific sugars in snap bean pods is possible. Heritability estimates may be partially inflated due to the limited number of years used in this study. Significant interactions would reduce heritability estimates; however, no significant genotype × year interactions were observed in this study. High heritability estimates of seed sucrose concentration were observed in soybean populations with similar experimental designs, suggesting sucrose concentrations are in general a highly heritability trait (Kim et al., 2005; Maughan et al., 2000).

Highly significant correlations were observed among pod fructose, glucose, and sucrose concentrations (Fig. 1). Fructose and glucose concentrations were positively correlated ($r = 0.79$). In contrast, both fructose and glucose concentrations were negatively correlated with pod concentration of sucrose, with $r = -0.70$ and -0.54, respectively. A negative correlation between the monosaccharides fructose and glucose and the disaccharide sucrose is consistent with the breakdown of sucrose to its monomers glucose and fructose by acid invertase (Sung et al., 1994). This observation is consistent with the contrasting effects observed for the QTL associated with RAPD marker W9.1050 (Fig. 2 and 3). In contrast, if the relationship between the monosaccharides and disaccharide were due to a phloem transporter the expected correlation would be positive. As sucrose levels transported through the phloem into the pod either increased or decreased, fructose and glucose levels would follow a similar pattern.

Table 1. Analysis of variance, year means, variance components, and narrow-sense heritability (h^2) for pod concentration of three sugars in an Eagle × Puebla 152 recombinant inbred line bean population.

		Mean squares ANOVA†‡		
		Pod sugar concentration (mg g^{-1} dry wt.)		
Source	df	Fructose	Glucose	Sucrose
Year (Y)	1	6951.29***	70.98	144.28***
Replication (year)	2	313.69	149.04**	7.74
Genotype (G)	73	924.29***	223.43***	25.25***
Y × G	73	143.84	35.59	5.07
Error (σ^2)		138.35	28.00	4.50
Year means§				
2009		44.39	22.18	7.72
2010		54.43	23.39	6.32
Variance components				
σ^2_g (G)		195.11	46.96	5.05
σ^2_{gy} (G × Y)		2.75	3.79	0.29
Heritability¶				
$h^2 \pm SE(\sigma^2_g)$		0.85 ± 0.16	0.85 ± 0.16	0.81 ± 0.16

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

†Measurements made in sieve size 4 pods (8.33–9.52 mm in diameter).

‡Mean square values are from Box-Cox transformed data (Box and Cox, 1964).

§Least square means from untransformed data.

¶h^2, narrow-sense heritability, = $\sigma^2_g/[\sigma^2/(ry) + \sigma^2_{gy}/y + \sigma^2_g]$, and SE($h^2$), standard error of heritability, = $SE(\sigma^2_g)/[\sigma^2/(ry) + \sigma^2_{gy}/y + \sigma^2_g]$, in which r = replications within years, y = years, and $SE(\sigma^2_g) = \{(2/(ry)^2([MS(G)/n + 1] + MS(Y \times G)/[(y - 1)(n - 1) + 2])\}^{1/2}$ with n = no. of families.

Mapping QTL for Sugar Concentrations in the Eagle × Puebla 152 RIL Population

The previously published RAPD map for the EP-RIL population (Skroch, 1998) was used to investigate putative QTL associated with variation in fructose, glucose, and sucrose concentration. Because no year × genotype interactions were detected, LSMEANs for each RIL were used to search for putative QTL. The RAPD marker W9.1050 on linkage group B1, inherited from Eagle, was associated with a QTL that results in increased fructose and glucose and decreased sucrose concentrations (Fig. 2 and 3; Table 2). The marker W9.1050 explained 25% of the variation in pod fructose concentration and 23% of the variation in pod sucrose concentration. In addition, RAPD marker F8.500 located on linkage group B6, also inherited from Eagle, was associated with a QTL that resulted in decreased pod glucose concentration (Fig. 3; Table 2). The markers W9.1050 and F8.500 associated with the two-QTL model explained 22% of the observed variation for glucose. No significant interaction was observed between RAPD markers W9.1050 and F8.500 for pod glucose concentration. Compared to previous studies that identified multiple QTL associated with sugar concentration in tomato, sweet corn, and soybean, a single-QTL model for pod fructose and sucrose concentrations and a two-QTL model for pod glucose concentration was unexpected (Fulton et al., 2002; Maughan et al., 2000; Qi et al., 2009).

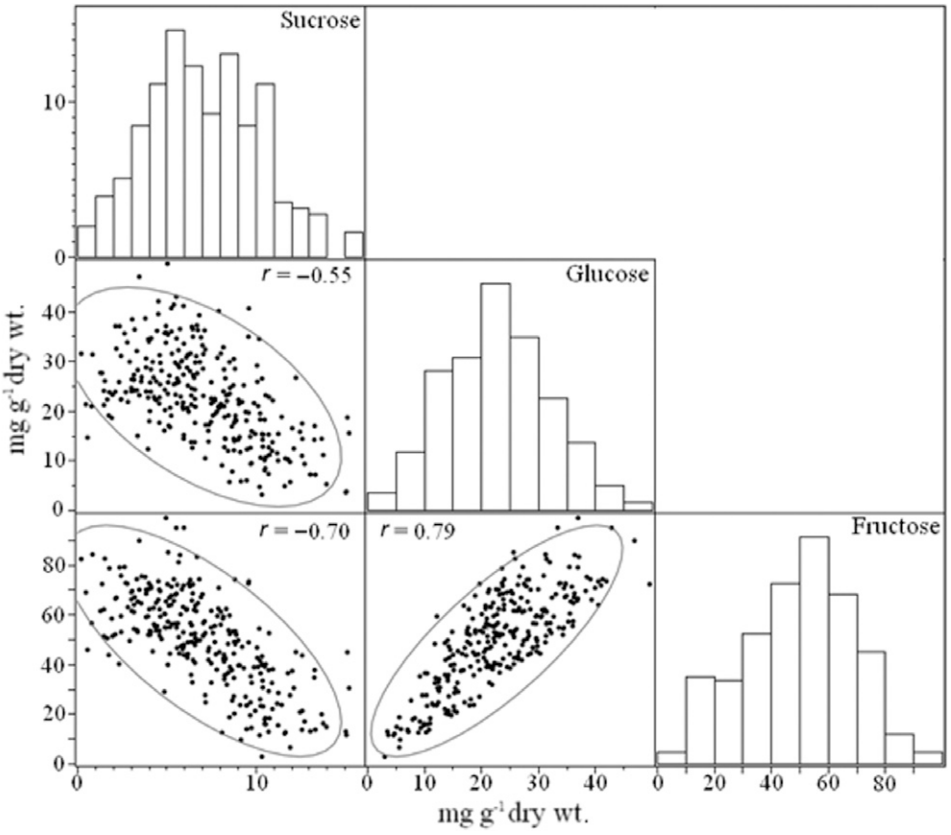

Figure 1. Correlations and distributions of Box-Cox transformed (Box and Cox, 1964) sucrose, glucose, and fructose concentrations in an Eagle × Puebla 152 recombinant inbred line bean population. Sugars were normally distributed.

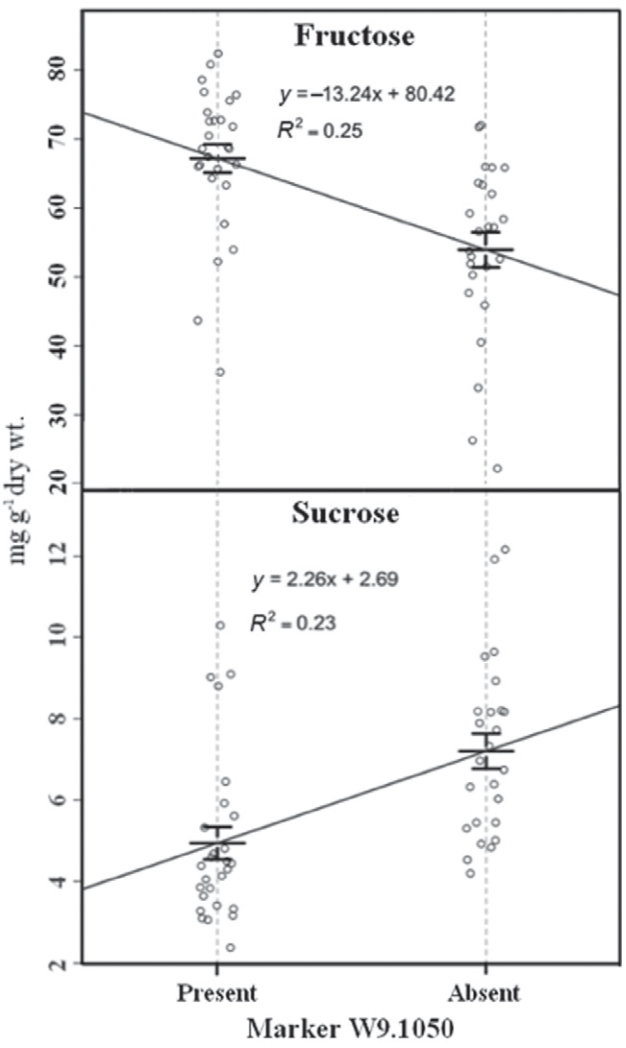

Figure 2. Effect plots of marker W9.1050 for fructose and sucrose concentration, linked to putative quantitative trait loci, in an Eagle × Puebla 152 recombinant inbred line bean population. Genotype means are plotted as circles. Standard errors are presented as short black bars.

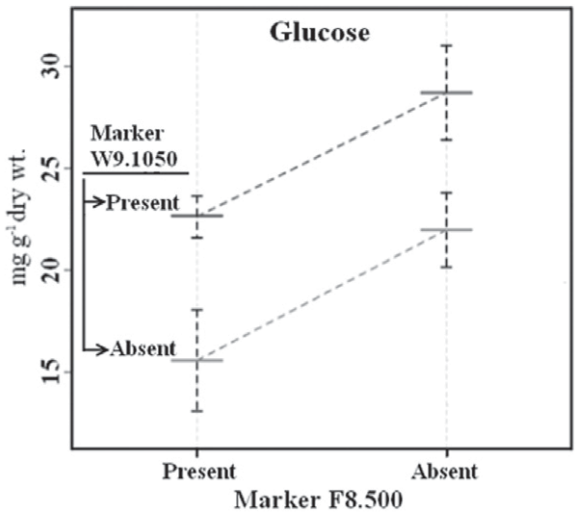

Figure 3. Effect plot of marker W9.1050 and F8.500 for glucose concentration, linked to putative quantitative trait loci, in an Eagle × Puebla 152 recombinant inbred line bean population. Genotype means are presented as long gray bars. Standard errors are presented as short black bars.

The smaller number of detected QTL for sugar concentration in the EP-RIL population compared to other populations may be a result of the smaller size of the EP-RIL population and the moderate number of molecular markers. An increase in the number of molecular markers or RILs may increase the ability to resolve additional QTL associated with sugar concentration.

The identification of a single QTL associated with all three sugars on linkage group B1 is consistent with the expected pattern of inheritance of a single locus with major effects and is also consistent with the reported activity of acid invertase (Sung et al., 1994). The presence of marker W9.1050 on linkage group B1 positively effects fructose and glucose concentrations while negatively effecting sucrose concentration. This effect duality suggests that the putative QTL associated with RAPD marker W9.1050 may be involved in the breakdown of sucrose into its monomers fructose and glucose either directly, for example, acid invertase, or indirectly, for example, a regulatory mechanism or a sucrose transporter. While other mechanisms may contribute significantly to the overall accumulation of sugars in developing snap bean pods, acid invertase plays a key role in the breakdown of sucrose into fructose and glucose during rapid pod growth before seed set and may be a candidate gene associated with the QTL on linkage group B1.

Table 2. Random amplified polymorphic DNA (RAPD) molecular markers associated with putative quantitative trait loci (QTL) in an Eagle × Puebla 152 recombinant inbred line (EP-RIL) population.

	QTL discovery in EP-RIL population						
Trait	RAPD marker (origin)	RAPD marker sequence 5′-3′	LOD score†	R^2	Effect value‡	Linkage group	Position (cM)
Fructose concentration	W9.1050 (Eagle)	*GTGACCGAGT*	4.57	0.25	8.61	1	45.4
Glucose concentration			4.28	0.12	3.98	1	45.4
Sucrose concentration			4.16	0.23	–1.44	1	45.4
Glucose concentration	F8.500 (Eagle)	*GGGATATCGG*	3.57	0.10	–4.51	6	23.9

†LOD, logarithm to the base 10 of the likelihood odds ratio, scores for glucose concentration trait from two-QTL model, y ~ Q1 + Q2.

‡Effects for presence of RAPD marker.

Further experimentation should be done to assess the genetic mechanisms associated with the QTL on linkage group B1. Sugar concentrations are an important component of flavor and consumer acceptance in fresh and processed snap beans. The identification of a gene or gene complex responsible for increased sugar concentrations may help a breeding program more efficiently develop new cultivars with different patterns of sugars. The high heritability of the QTL associated with sugars suggests that selection based on pod sugar content will be an effective means of breeding for modified pod sugar concentrations.

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